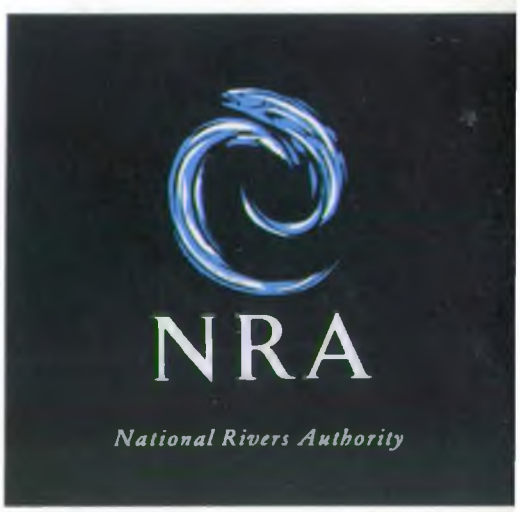
Discharge Control by Direct Toxicity Assessment (DTA)

Interim Protocol

WRc plc



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DISCHARGE CONTROL BY DIRECT TOXICITY ASSESSMENT (DTA) - INTERIM DOCUMENT

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SUMMARY

I OBJECTIVES

This report aims to:

(1) Consider if a wider use of Direct Toxicity Assessment (DTA) to assess and control discharges would be beneficial to the management of UK surface water quality.

(2) Examine the problems of applying such an approach.

(3) Outline a draft protocol for the application of DTA in discharge control.

(4) Identify a strategy for assessing and refining the draft protocol.

II REASONS

Traditionally, control of discharges to surface waters has been achieved by establishing maximum permitted concentrations of potentially-polluting substances in the effluent, and undertaking sampling and chemical analysis to ensure that those concentrations are not exceeded.

In recent years, however, there has been increasing interest in the application of DTA to evaluate and control effluent quality - and, specifically, for the establishment and monitoring of Discharge Consent Conditions (DCCs) expressed in terms of toxicity; ie Toxicity-Based Consents (TBCs).

Because there is extensive interest in the use of DTA on the part of regional pollution control staff, and direct experience of TBCs in several UK regulatory agencies, this document was distributed in draft for comment, and a number of amendments made as a result. This interim protocol will now be collaboratively tested by WRc and regulatory agencies.

III CONCLUSIONS

(1) Direct Toxicity Assessment has a potentially important role in the control of polluting discharges to UK surface waters.

(2) It is complementary to, rather than a substitute for, conventional chemical-specific controls. For discharges containing a limited number of well-known substances, for which toxicological data are available, chemical control will be adequate and cost-effective.

(3) The use of DTA will be advantageous for discharges of substances for which suitable toxicological data are not available, and for complex and/or variable discharges where chemical and biological interactions are likely to vitiate the chemical-specific approach.

(4) The wider use of DTA necessitates the development of a protocol for its effective and efficient application, and the establishment of quality control systems to ensure consistency of the data obtained.

(5) Adoption of DTA should not draw effort away from the acquisition of toxicological data for specific contaminants, upon which sound EQSs may be based.

IV RECOMMENDATIONS

It is recommended that the regulatory agencies should adopt Direct Toxicity Assessment as a routine part of their pollution control strategies, and that the interim protocol described should be tested collaboratively by WRc and regional pollution control staff.

V RESUME OF CONTENTS

Current UK pollution control practices are described, and the advantages and disadvantages of both the chemical-specific and DTA approaches are examined. Existing experience of DTA and toxicity reduction procedures (particularly in the United States) is considered, and the potential role of DTA defined.

An interim protocol for the use of DTA in UK surface water quality management is outlined in the main text, and developed in greater detail in an Appendix to it.

CONTENTS

APPENDIX

SECTION 1 - INTRODUCTION

Traditionally, control of potentially-polluting discharges to surface waters (fresh and saline) has been achieved by establishing maximum permitted concentrations of relevant polluting substances in the effluent, and undertaking appropriate sampling and chemical analysis of the effluent to ensure that those concentrations are not exceeded.

There is, however, increasing interest in the application of Direct Toxicity Assessment (DTA) to evaluate and control effluent quality, and in the establishment of Discharge Consent Conditions (DCCs) expressed in terms of toxicity - ie Toxicity-Based Consents (TBCs). It is the purpose of this report to:

(1) Consider whether or not a wider use of DTA to assess and control discharges would be beneficial to the management of UK surface water quality.

(2) Examine the problems of applying such an approach.

(3) Outline an interim protocol for the application of DTA in discharge control.

(4) Identify a strategy for assessing and refining the interim protocol.

It is recognised that there is extensive interest in the use of DTA on the part of regional pollution control staff in the water industry, and direct experience of the setting of Toxicity-Based Consents in several UK regulatory agencies. It is therefore intended that the interim protocol will be subjected to collaborative testing by WRc and local regulatory agencies - testing which will include a number of case studies involving discharges to both fresh and saline waters.

It is outside the scope of this report to consider who should undertake the tests, and who should pay for them. We presume, however, that the regulatory agency will determine in which laboratory the tests are to be undertaken, and that the ability to pass costs on to the discharger will apply to DTA in the same way as it does for chemical monitoring.

SECTION 2 - CURRENT UK POLLUTION CONTROL PRACTICE

Although the UK is committed to a precautionary approach to pollution control, through application of Best Available Technology Not Entailing Excessive Costs (BATNEEC) to industrial discharges, this is to be applied in conjunction with the established Environmental Quality Objective/Environmental Quality Standard (EQO/EQS) approach, which has long been the cornerstone of UK pollution control practice. In essence, the implications of both the BATNEEC and the EQO/EQS approaches are to be assessed for each significant discharge, and the more stringent resulting Discharge Consent Condition applied (Water Authorities Association 1988). Here, we shall be concerned with comparing the EQO/EQS and DTA approaches; the BATNEEC approach will not be considered further, although its overall role in discharge control must be borne in mind throughout.

Under the EQO/EQS approach, a range of EQOs are set to protect legitimate Uses of receiving waters; such EQOs include general ecosystem protection as well as the maintenance of quality for such purposes as potable abstraction. An EQO is considered to be met if the water in question complies with a range of EQSs relevant to the Use concerned. Discharge Consent Conditions are set (usually in terms of both concentration and load) so that the relevant EQSs are met in the waters receiving the discharge, beyond the boundary of a specified Mixing Zone (MZ).

In other words, the EQSs applicable to the most demanding EQO for the receiving waters, taken in conjunction with the location and size of an acceptable MZ, normally determine the maximum permitted concentrations of relevant substances in the effluent itself. (Note, however, that there is also a requirement that any region of acute toxicity adjacent to the discharge be minimised - subject to the proviso of excessive costs - and this, rather than compliance with the EQS beyond the Mixing Zone boundary, could sometimes be the factor which determines the stringency of the DCC.)

In the case of specific Uses such as potable water abstraction, the EQSs are obtained from relevant European Community (EC) legislation. For general ecosystem protection, with which ve are here concerned, the EQSs are either:

(a) For List I substances, those specified in relevant EC Directives,

or

(b) For List II substances, those determined by the UK itself, from available toxicological data with appropriate application factors[1].

SECTION 3 - ADVANTAGES AND DISADVANTAGES OF THE CHEMICAL-SPECIFIC APPROACH

The EQO/EQS approach is satisfactory for simple effluents of well defined and consistent composition, containing only toxicants for which there are adequate toxicological data on which to base the EQS.

It has the advantage of leading to simple, clear-cut DCCs, compliance with which can be assessed by chemical analysis of the effluent. Chemical analysis - particularly for "traditional" contaminants - is both relatively inexpensive and, if appropriate Analytical Quality Control (AQC) is exercised, capable of adequate accuracy and comparability.

There are, however, a number of disadvantages to an approach which is entirely chemical-specific[2,3,4,5,6,7]:

1. Many effluents contain organic chemicals which are not readily or accurately identifiable or measurable by even the most sophisticated analytical techniques available to routine monitoring laboratories.

2. Even if chemical data are obtainable, toxicological data upon which to set EQSs are sparse or unavailable for many thousands of

synthetic chemicals. Furthermore, such information as is available on toxicity may not be relevant to local organisms.

3. These problems may be exacerbated by the highly complex composition of effluents from modern chemical plants, which can:

   (i) Make it costly to measure accurately all the chemicals present (assuming it is even possible to do so - see (1) above).

   (ii) Cause problems in applying EQSs, which are derived on the basis of single-substance toxicity. (EQSs do not take account of the possible chemical interactions between different discharge components, and with constituents of the receiving waters, both of which may affect overall toxicity; nor do they allow for the possible synergistic and antagonistic toxicological effects of substances in complex discharges.)

4. The difficulties may be further compounded by the variable composition of many complex effluents, particularly those from plants operating batch processes.

SECTION 4 - ADVANTAGES AND DISADVANTAGES OF DIRECT TOXICITY ASSESSMENT (DTA)

In principle, some or all of the above disadvantages of the conventional, chemical-specific approach can be overcome - in whole or in part - by an approach involving the application of DTA, which considers the effects on organisms of each effluent as a whole[2,5,6,7]. Thus, in principle, the use of DTA can:

1. Detect the biological effect of the combination of all compounds present, even if they cannot be identified and/or measured by chemical means.

2. Control the toxicity of an effluent which contains substances for which no toxicological data are available.

3. Address complex effluents without the cost increasing with the number of substances present, and with due account of chemical and toxicological interactions.

4. Cope with variations in the composition of complex effluents.

However, in practice, the biological approach itself has a number of disadvantages:

1. It is relatively expensive and time-consuming, in comparison with chemical assessment, if there are only a small number of chemical determinands, or if the chemicals concerned are easily measured.

2. Because the identity and importance of individual toxicants is not revealed, the approach:

(i) lends itself less readily than does chemical-specific control to toxicity reduction of the effluent,

(ii) does not provide information on the properties (eg bioaccumulation potential) of specific substances, and

(iii) may not cover toxicity released downstream by reaction of effluent components.

3. It requires facilities and expertise which, unlike those for the basic chemical measurements, are not available in every UK pollution control laboratory; moreover, quality control procedures for toxicity testing are less well developed than those for chemical analysis.

4. The results of toxicity testing are, at present, less familiar to pollution control staff than those of chemical testing.

5. The chemical-specific approach can be more readily related to the current UK river classification system, which relies exclusively on chemical measures of quality.

None of these is an overwhelming reason for not pursuing the approach. Point (1) indicates that DTA should not be applied when the simpler chemical approach will suffice. Nor is 2(i) insurmountable - toxicity reduction can be accomplished using toxicity testing in conjunction with physico-chemical fractionation procedures and/or laboratory simulations of possible waste treatment processes (see Mount[2] and below). With regard to points 2(ii) and 2(iii), the chemical-specific approach would not give this information either, if analytical difficulties precluded identification of the toxic species.

Points (3) and (4) require the development of appropriate techniques and the education of relevant staff in their application. Finally, with regard to point (5), there is widespread agreement - and intention - that biological information should play an important part in future classification systems; the wider use of DTA is, therefore, entirely congruent with current views on assessment of water quality.

SECTION 5 - EXISTING EXPERIENCE OF DIRECT TOXICITY ASSESSMENT

The United States has a well-developed programme of biological testing for the control of discharges to surface waters. The historical development of this programme has been described by Wall and Hanmer[7] of the US Environmental Protection Agency (EPA).

Toxicological control of effluents was introduced through the National Pollution Discharge Elimination Scheme (NPDES) which is based, as in the UK, on use-oriented environmental quality standards, and is controlled by the individual States' Departments of Environmental Protection.

The Agency began a programme of research in the mid-1970's to develop both acute and chronic toxicity tests for effluents, using a fish, an invertebrate and an alga. Following this, a programme of effluent testing was initiated in 1981 to assess the ability of the tests to predict the effects of complex effluents upon receiving waters. On the basis of work on eight multiple-discharge situations, EPA decided that

toxicity tests with three to five species did predict receiving water effects and could be used to control toxic discharges.

Formal introduction of toxicity-based controls took place in 1984, by means of a national policy statement recommending their use in combination with chemical controls[8]. In 1985, the Agency published a manual giving detailed guidance on the regulatory application of biological testing[5]; by 1987, Wall and Hanmer[7] were able to report that toxicity testing requirements had been written into over 1400 industrial discharge permits (ie consent conditions), representing about 38% of the permitted major industrial discharges, and into about 400 municipal (ie sewage) treatment works permits, representing about 10% of the major permitted municipal discharges.

It was concluded that toxicity testing - as an adjunct to chemical analysis - improved the assessment and control of potentially-polluting discharges, and that EPA would press for increased use of the approach[7].

A number of other countries have reported their experiences of DTA, including Canada, Eire, Finland, France, Norway, Sweden and the United Kingdom[9], see also Bengtsson[10] for details of a US-Swedish collaborative project). Although none of the programmes described seems comparable in scope and development with that of the United States, there is general agreement that DTA is a valuable addition to chemical control of discharges; this is also reflected in the production of general guidance on the use of biological testing for water pollution assessment and control by the Organisation for Economic Cooperation and Development[11].

Although UK experience of DTA is limited in comparison with that of the United States (see, for example, the review of early work by Pearce[12]), there is growing interest in the approach. Recent applications include:

i) the use, by Welsh Water pollution control staff, of the Microtox bacterial test to assess the toxicity of a number of discharges to

estuarine and coastal waters (personal communication from Dr C Pattinson, Welsh Water),

ii) the application, by Clyde River Purification Board, of toxicity testing to discharges from a pharmaceutical plant to tidal waters and from an explosives factory to a river[13,14].

SECTION 6 - TOXICITY REDUCTION

As noted previously, one of the major perceived disadvantages of DTA is the fact that it does not identify the chemicals causing toxic effects, and so makes it more difficult to reduce the toxicity of effluents.

Mount[2] has challenged this view, pointing out that BOD and Suspended Solids removal has not demanded detailed knowledge of the specific components involved, and that EPA is similarly committed to limiting toxicity without expending the effort required to identify specific causal agents. A similar point has been made by other EPA staff[7]. Three basic approaches to toxicity reduction can be envisaged:

(1) The Causative Agent approach, in which detailed chemical analysis is brought to bear on a problem revealed by toxicity testing. Once specific causal agents are identified, treatment or substitution options can be assessed.

(2) The Fractionation approach, in which toxicity testing is applied to fractions of the effluent separated by physico-chemical means (eg volatiles, acid extractables) to trace toxicity to a specific physico-chemical fraction without attribution of effects to specific compounds.

(3) The Toxicity Treatability approach, in which different treatment options are applied on a bench scale and their efficacy assessed by toxicity testing.

Mount[2] prefers approach (3) - which he calls "waste engineering fractionation" to approach (2), but the writer suspects that, in practice, all three approaches will be useful; which will be most fruitful in a particular case will depend on the complexity of the effluent and the nature of its components.

Wall and Hanmer[7] report that recent EPA experience favours the Causative Agent approach, because it is more effective to keep a substance out of a waste-stream in the first place than to treat the stream after contamination. They also point out that, even if a unique attribution of toxicity is not achieved, the specific process in a complex plant which contributes the causative toxicant(s) may be identified, such that only its waste-stream - rather than the entire effluent - need be treated.

SECTION 7 - THE ROLE OF DIRECT TOXICITY ASSESSMENT

From the above, we conclude that:

(1) Direct Toxicity Assessment has a potentially important role in the control of polluting discharges to UK surface waters, both fresh and saline.

(2) It should be seen as complementary to, rather than a substitute for, conventional chemical-specific controls; it is not a panacea. For discharges containing a limited number of well-known substances, for which suitable toxicological data are available, chemical-specific control will usually be quite adequate - and more cost-effective.

(3) The use of DTA will be particularly advantageous for discharges containing substances for which suitable toxicological data are not available, and for complex and/or variable discharges where chemical and biological interactions are likely to vitiate the chemical-specific approach.

(4) The wider use of DTA necessitates the development of a general protocol for its effective and efficient application. It also requires the establishment of quality control systems to ensure that the data obtained are adequately consistent. Otherwise, the confidence of dischargers, environmental groups and the public will be undermined, and the full benefits of the approach lost.

(5) Adoption of DTA should not draw effort away from the planned acquisition of toxicological data for specific contaminants, upon which sound EQSs may be based; otherwise, the effectiveness of chemical-specific control will be impaired.

SECTION 8 - THE PROPOSED UK APPROACH

It is clear that considerable effort would be involved in establishing DTA as a routine element of surface water pollution control in the UK. Whilst a number of organisations have experience of the techniques involved, application is patchy, there is no uniformity of approach and the overwhelming majority of discharges are subject only to chemical-specific controls.

Experience of DTA is greatest in the United States, where a substantial proportion of consented discharges are now subject to toxicological control as an adjunct to chemical analysis. However, given the limited penetration of DTA in the UK to date, it would not seem cost-effective simply to adopt the US EPA protocols[5], which specify the use of acute toxicity tests on as many as three aquatic organisms at the screening stage. The costs of this, and the absence of suitable facilities in many parts of the UK regulatory system, would impede adoption of DTA and thereby delay the benefit of improved pollution control.

It is therefore proposed that a much simpler, commercially-available toxicity test - the Microtox system (Beckman Instruments Inc - see, for example, Bulich[15]) - be used for initial assessment of those effluents thought likely, on currently available information, to be suitable candidates for DTA. Because the Microtox system is a

commercial test, there will inevitably be concern about the long-term availability of the system. It is therefore recommended that the Microtox system be used for the time being, but that consideration be given to the possible development of another simple, rapid and inexpensive test specifically for the UK regulatory agencies, the continued availability of which would be guaranteed. (Although the Microtox bacterium may not respond to toxicants associated with suspended particulate matter, the same is likely to be true for all rapid screening tests. The Microtox system can be affected by the colour of samples, but offers facilities to correct for such an effect.)

As a result of Microtox screening, discharges would be placed in four categories depending, principally, on their potential toxic impact on the receiving waters. (Although the Microtox test is of acute toxicity, an estimate would be made of possible chronic toxicity in the receiving waters using an application factor to allow for inter-species differences in sensitivity and an assumed acute-to-chronic ratio.)

Category A discharges, which screening shoved might cause chronic toxicity in their receiving waters, would be subjected to detailed investigation, typically leading to toxicity or discharge reduction and a full Toxicity-Based Consent condition (TBC). The latter would normally be based on acute toxicity testing with three representative organisms, with routine monitoring using the Microtox system wherever possible. The representative organisms could be such species as (for freshwater) the invertebrate, Daphnia magna, the rainbow trout, Salmo gairdneri, and the alga, Chlorella vulgaris, and (for marine waters) the brown shrimp, Crangon crangon, juvenile plaice, Pleuronectes platessa (or turbot, Rhombus maximus) and the queen scallop, Chlamys opercularis[13]. Other possibilities for discharges to marine waters include tests using the larvae of the common mussel, Mytilus edulis or Pacific oyster, Crassostrea gigas[16] and an algal test (eg using Phaeodactylum tricornutum[17]). A test using rainbow trout (Salmo gairdneri) may also be appropriate to safeguard the passage of migratory salmonids in estuaries.

(It is arguable that more representative species could be chosen; for example, Gammarus pulex might be a better representative of riverine invertebrates than Daphnia magna and the indigenous brown trout, Salmo trutta, a better choice for the fish test than the rainbow trout, Salmo gairdneri. However, the use of such organisms would require further development work to ensure that the reliability of results matched that of the results of the more common tests; it is not considered that implementation of Direct Toxicity Assessment need await such work. In general, the aim should be to use a relatively small number of well-established tests. However, this aim may need to be tempered in specific cases by the desire for information on sensitive local species.)

Category B discharges, showing intermediate toxicity, would not be subjected to immediate toxicity reduction. Full Toxicity-Based Consents would be set, as outlined above, and the discharges concerned would be investigated further after those in Category A had been dealt with satisfactorily.

Category C discharges, showing low toxicity, would be given a simpler Microtox-based TBC if they were of very variable composition; otherwise, their control would continue to be undertaken using chemical-specific consent conditions alone.

Category D discharges, showing little or no toxicity, would continue to be controlled by chemical-specific consent conditions alone.

Appendix A describes the interim protocol; whilst it does not follow the EPA approach in detail, it has been drawn up with the benefit of practical experience in the USA, described in the relevant EPA manual[5]. As noted above, the EPA approach to toxicity screening has not been followed exactly, but many other aspects of the protocol are similar to their EPA counterparts, modified as necessary for UK conditions.

Following a meeting with regional pollution control staff to discuss the application of DTA, WRc will begin to establish, as part of its

1989/90 environmental research programme, both the quality control procedures for the necessary toxicity tests and the collaborative case studies to be carried out in various parts of the UK.

SECTION 9 - CONCLUSIONS

(1) Direct Toxicity Assessment (DTA) has a potentially important role in the control of certain types of polluting discharges to UK waters, as a complement to conventional chemical-specific controls.

(2) DTA is particularly advantageous when a discharge contains substances for which suitable toxicological data are lacking, and for complex and/or variable discharges. For discharges containing a limited number of well-known substances, chemical-specific control will usually be adequate and more cost-effective.

(3) Wider use of DTA requires the establishment of a UK-wide protocol (with adequate scope for case-specific modification), and of quality control systems to ensure that its results are accepted by dischargers, environmental groups and the public.

(4) Adoption of DTA should not, however, draw effort away from the planned acquisition of toxicological data for specific contaminants, upon which sound EQSs may be based.

(5) The interim protocol of Appendix A for achieving wider application of Direct Toxicity Assessment in the UK will be tested by collaborative studies involving WRc and regional pollution control staff.

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APPENDIX A - INTERIM PROTOCOL FOR ESTABLISHING DIRECT TOXICITY ASSESSMENT

INTRODUCTION

The following is an interim protocol for the phased introduction of Direct Toxicity Assessment (DTA) as a routine procedure for effluent characterisation and control in the United Kingdom. It is recognised that the control of discharges to surface waters presents case-specific complexities and difficulties, the solution of which often demands local adaptation of general principles. The approach described is therefore to be viewed as general guidance, to be varied as necessary to address specific cases.

The procedures described are summarised in Figure 1.

SCREENING

1. It would be wasteful of resources for Direct Toxicity Assessment to be introduced without a clear (albeit, inevitably, partially subjective) assessment of its likely value in controlling individual discharges. A 3-stage strategy involving preliminary discharge screening is therefore suggested:

Stage 1: Selection, in priority order, of all discharges with the potential to benefit from DTA, on the basis of an assessment of existing data and background information.

Stage 2: Application of one or more simple, acute toxicity tests to the selected discharges, in priority order determined by Stage 1, leading to:

Stage 3: (1) Further investigation of those discharges estimated to cause chronic toxicity in the receiving waters, with a view to toxicity and/or discharge reduction, probably

followed by establishment of a Toxicity-Based Consent (TBC).

(2) Establishment of full TBCs for those discharges showing intermediate toxicity.

(3) Establishment of Microtox-based TBCs for those discharges showing low toxicity, but whose composition is likely to be very variable.

(4) Continued reliance upon chemical-specific consent conditions for those discharges which:

(i) show little or no toxicity or
(ii) show low toxicity and limited variability of composition.

STAGE 1

2. The objectives of Stage 1 screening are to exclude from subsequent work those discharges for which existing, chemical-specific control is satisfactory, and to identify those discharges most urgently needing toxicological screening. The following factors should therefore be considered in selecting discharges for Stage 2 appraisal:

(a) Existing knowledge of the impact of the discharge upon receiving waters, and the class of the latter.

(b) The presence (or likely presence) in the discharge of substances potentially toxic to aquatic ecosystems, but not subject to EQSs, or for which toxicological data are lacking.

(c) The complexity of composition of the effluent.

(d) The magnitude of the discharge relative to the diluting capacity of the receiving water.

(e) Any available information on the toxicity of the whole effluent or of constituent waste streams.

3. It should be borne in mind that, in the US, prior beliefs about the relative toxicities of different types of effluent were often overturned by direct toxicity testing. No class of discharges should, therefore, be excluded completely at Stage 1 without conclusive evidence that chemical-specific control is adequate for them; otherwise, no check on this view would be obtained by toxicity testing. The better policy is to retain at least a limited number of representatives of each broad type of discharge for appraisal at Stage 2.

STAGE 2

4. The objectives of Stage 2 are to identify those discharges most urgently needing further assessment, probably leading to toxicity reduction and/or control by Toxicity-Based Consent. Acute toxicity testing using relevant aquatic species[18,19] and the Microtox system of luminescent bacteria[19] have both been suggested. Given the very limited application of Toxicity-Based Consents in the UK to date, it is suggested that the Microtox system is used as the principal screening test. The sensitivity of the photobacterium concerned to many toxicants and effluents is similar to that of numerous, more typical aquatic organisms[20,21], and the cost of the Microtox test is much less than that of conventional toxicity testing.

5. The test should normally be applied directly to dilutions of the effluent in the Microtox medium (rather than in upstream receiving water), to obtain an estimate of absolute effluent toxicity (but see also paragraph 12 on multiple sources). This will be in the form of an EC50 value, expressed as a percentage concentration of the effluent. Because this gives a measure

which is inversely related to the toxicity of the effluent, it is more convenient to express the Effluent (Microtox) Acute Toxicity (EMAT) in Toxic Units (TU), by dividing the percentage concentration EC50 into 100:

EMAT = 100 / EC50

In other words, an effluent with a percentage concentration EC50 of 1% contains 100 TU, whereas one with a percentage concentration EC50 of 25% contains only 4 TU.

6. Because Stage 2 involves estimating whether or not a discharge is likely to cause practically-important chronic toxicity in the receiving water ("In-Stream Toxicity"), it is recommended that the results of the Microtox testing should be combined with an Acute-Chronic Ratio (ACR), with an Application Factor (AF) for species sensitivity, and with a "Worst Case" Dilution Factor (WCDF). To do this, the Estimated Chronic In-Stream Toxicity (ECIST) is calculated, again in Toxic Units*:

ECIST = EMAT x ACR x AF / WCDF

An Acute-Chronic Ratio of 10 is suggested[5], and an Application Factor of 10 will normally be appropriate. (Note, however, that a higher Application Factor, up to 100, may be appropriate if the toxic components of the effluent are of high persistence and/or bioaccumulability. If this is suspected, special tests may be conducted and an application factor selected accordingly[13].)

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* Note that, although a toxic unit has previously been defined as the strength of a chemical as a proportion of its lethal threshold concentration[22], we are here choosing to extend this usage to cover chronic toxicity as well.

7. The Worst Case Dilution Factor can be obtained in a variety of ways depending on the nature of the discharges concerned. For discharges to rivers, it is simple to calculate the dilution of an average effluent flow by a low river flow. The 5 and 10 percentile weekly average river flows (7Q5 and 7Q10), used by EPA to assess chronic toxicity in stressed and unstressed receiving waters, respectively, seem quite appropriate. More sophisticated procedures[23] could be used to calculate percentiles of Estimated Chronic In-stream Toxicity, but their use does not seem justified for screening purposes.

8. For estuarine and coastal discharges, an estimate of the dilution achieved at the boundary of the Mixing Zone on a neap tide can be obtained by hydrodynamic and dispersion modelling[24]. Note, however, that a cruder estimate of dilution may be sufficient for screening purposes, and that the same reference has an Appendix dealing with the rough estimation of dilution in tidal waters.

9. Numerous criteria could be chosen for assessing the results of screening tests; the following approach is suggested:

   (1) Priority discharges (Category A) are those giving Estimated Chronic In-Stream Toxicities exceeding 1.0 TU. They should be investigated further, with a view to toxicity and/or discharge reduction, probably followed by the establishment of a full Toxicity-Based Consent as described in paragraph 17 (unless toxicity is reduced to such an extent that the discharge, on re-testing, falls into Category C or D - see below).

   (2) Category B discharges are those giving Estimated Chronic In-Stream Toxicities above 0.1 TU, but not exceeding 1.0 TU. They should be reserved for further investigation after the priority cases; pending such investigation, a full Toxicity-Based Consent should be set (paragraph 17).

(3) Discharges (Category C) with Estimated Chronic In-Stream Toxicities above 0.01 TU, but not exceeding 0.1 TU, may be set a Toxicity-based Consent in terms of the Microtox test alone, or subjected only to a chemical-specific consent condition. The choice should depend upon knowledge of the likely variability of the effluent composition; the more variable the composition, the more appropriate is a Toxicity-Based Consent.

If a Microtox TBC is set, the consented Effluent (Microtox) Acute Toxicity will normally be obtained using the equation of paragraph 6, setting the Estimated Chronic In-Stream Toxicity equal to 1 TU (but see also paragraph 12 on multiple sources) and the Acute Chronic Ratio and Application Factor each equal to 10, such that:

EMAT = WCDF / 100

Exceptionally, an Application Factor of 100 will be more appropriate - see paragraph 6.

(4) Discharges (Category D) with Estimated Chronic In-Stream Toxicity less than 0.01 TU should be consented on the basis of chemical-specific consent conditions alone.

Once all Category A discharges have been dealt with by toxicity and/or discharge reduction and, where appropriate after such reduction (see paragraph 16), by establishment of a Toxicity-Based Consent, a similarly detailed assessment should be carried out on Category B discharges, in order of their potential toxic impact. In this way, unsuitability of the Acute-Chronic Ratio and/or Application Factor chosen for screening will not result in continued neglect of a discharge having an important effect on receiving waters.

10. Notwithstanding the reasons given above for using the Microtox as the principal screening test, there may be circumstances where a more detailed initial screening is called for. Thus, for example, acute testing using an alga (eg Chlorella vulgaris[25,26]), would be an appropriate adjunct to Microtox testing for the effluent from a herbicide factory, testing with an invertebrate (eg Daphnia magna[25,27]), would be similarly appropriate for an insecticide works, and a fish test (eg using rainbow trout, Salmo gairdneri[25,27]), would be advisable for a discharge to an important fishery. (The above are all, of course, appropriate for discharges to fresh waters; a similar approach using appropriate marine organisms - see main report for examples - could be adopted for discharges to tidal waters.) A combination of all three additional types of test might be prudent for a particularly important discharge suspected of having a major impact on its receiving water body.

11. Whenever such tests are performed, simultaneous use of the Microtox test is recommended to build up a comparison of the responses of the various tests to different types of effluent. (WRc is also currently investigating the comparative toxicities of a range of chemicals to the Microtox bacteria, Daphnia and fish). In determining the action to be taken in such cases, the criteria of paragraph 9 should be applied to the results of the most sensitive test undertaken.

12. The procedures described above are applicable to those cases where only a single discharge affects the receiving waters. In cases where two or more discharges exert an important effect, the allocation of allowed toxicity load from each will need to be reduced to achieve an absence of chronic toxicity outside the relevant Mixing Zones. One approach is to use the absolute toxicity data and estimated dilution factors for all the discharges concerned to estimate the toxicity each contributes to the receiving water of the others, and thereby derive an equitable apportionment of diluting capacity. This may be accompanied by the assessment of relative, as well as absolute,

effluent toxicity, involving the use of upstream receiving water - rather than just pure water - in preparing the Microtox dilution medium. It may also be prudent to carry out ambient chronic toxicity tests on the receiving waters themselves using suitable species[5].

STAGE 3

13. In Stage 2, discharges were ranked according to their potential for causing chronic toxic effects in the receiving water, as estimated from Microtox testing and a knowledge of "worst-case" dilution. The most toxic (Category A) discharges - those having Estimated Chronic In-Stream Toxicities exceeding 1 TU - require effluent toxicity and/or discharge reduction, probably followed by the setting of a Toxicity-Based Consent.

14. Three basic approaches to toxicity reduction may be employed:

    (1) The Causative Agent approach, in which detailed chemical analysis is brought to bear on a problem revealed by toxicity testing. Once specific causal agents are identified, treatment or substitution options can be assessed.

    (2) The Fractionation approach, in which toxicity testing is applied to fractions of the effluent separated by physico-chemical means (eg volatiles, acid extractables) to trace toxicity to a specific physico-chemical fraction without attribution of effects to specific compounds.

    (3) The Toxicity Treatability approach, in which different treatment options are applied on a bench scale and their efficacy assessed by toxicity testing.

    It is probable that all three approaches may be useful in practice, the most fruitful in a particular case depending

on the complexity of the effluent and the nature of its components. It should be noted, however, that EPA experience favours the Causative Agent approach, because it is more effective to keep a substance out of a waste-stream in the first place than to treat the stream after contamination.

15. After completion of toxicity reduction, the Effluent (Microtox) Acute Toxicity is re-evaluated and judged acceptable if it results in an Estimated Chronic In-Stream Toxicity less than 1 TU (subject, of course, to the conditions of BATNEEC and to any special requirements arising from multiple discharges). If toxicity reduction alone cannot achieve this, discharge reduction will need to be considered, and the Estimated Chronic In-Stream Toxicity achieved by such reduction recalculated using the new Worst Case Dilution Factor and judged against the criterion of 1 TU.

16. Folloving toxicity and/or discharge reduction, the further action to be taken will be determined in accordance with paragraph 9, using the revised Effluent (Microtox) Acute Toxicity and/or Worst Case Dilution Factor, again with Acute-Chronic Ratio and Application Factor values of 10 (or, exceptionally, an Application Factor of 100 - see paragraph 6). Thus, for example, if the toxicity or effluent reduction brings about an Estimated Chronic In-Stream Toxicity of less than 0.01 TU, chemical-specific control can be employed without a Toxicity-Based Consent. On the other hand, a reduction of the Estimated Chronic In-Stream Toxicity only to 0.3 TU would necessitate the establishment of a full TBC - see paragraph 17.

17. For those discharges found (on initial testing, or after subsequent toxicity or discharge reduction) to have an Estimated Chronic In-Stream Toxicity of 0.1 to 1.0 TU, a

full Toxicity-Based Consent should be set. The following general procedure is proposed:

(a) On a number of occasions (to be judged in relation to the expected variability of effluent composition, but not less than 4 over a minimum period of 3 months), the absolute acute toxicity of the effluent shall be assessed by tests with Daphnia magna[25,27], rainbow trout, Salmo gairdneri[25,27], and the alga Chlorella vulgaris[25,26], together with the Microtox system. (These all refer to a discharge to fresh water; see main text for a selection of appropriate marine species.)

(b) The results for the most sensitive of the three named species shall be used to "calibrate" the Microtox results, such that a consented Effluent (Microtox) Acute Toxicity can be set, equivalent to an Estimated Chronic In-Stream Toxicity of 1 TU for the most sensitive species (but see also paragraph 12 on multiple sources). In this way, the simple and relatively inexpensive Microtox test, which can be used by the discharger himself, can be used for routine monitoring.

(c) Routine monitoring, by the discharger and the regulatory agency, can then be carried out using the Microtox system, with the proviso that testing using the most sensitive of the three species named above shall be periodically repeated, and the Effluent (Microtox) Acute Toxicity adjusted by "recalibration". The frequency with which such recalibration is required should be determined on a case-by-case basis, account being taken of:

(1) The closeness of the results of routine monitoring to the consented Effluent (Microtox) Acute Toxicity.

(2) The expected variability of discharge composition.

(3) Changes in the nature and/or operation of the plant.

(4) The importance of the discharge to the receiving water quality.

It is suggested that recalibration should be undertaken at least once every 3 and 5 years, respectively, for Category A and B discharges.

(d) In the case of particularly important discharges, it may be appropriate to supplement the acute toxicity tests described in (a) above by chronic toxicity tests - for example, a test of Daphnia reproduction. In this way, a direct assessment of chronic toxicity can be used to calibrate the Microtox without the assumption of an Acute-Chronic Ratio.

(e) If the Microtox test proves insufficiently sensitive for use as a monitoring technique, attention will need to be given to the use of one of the other tests using aquatic organisms for that purpose. Given the greater complexity and cost of such tests, the frequency of monitoring may need to be re-assessed.

18. As noted in the main report, there is a requirement for any region of acute toxicity within the Mixing Zone to be minimised, within the constraint of "reasonable cost". Attention must be paid to this, as well as to the avoidance of chronic toxicity outside the Mixing Zone. The suggested approach is to use the Effluent (Microtox) Acute Toxicity, with an Application Factor of 10, to assess what Critical Initial Dilution Factor (CIDF) would give a level of acute toxicity less than 0.3 TU:

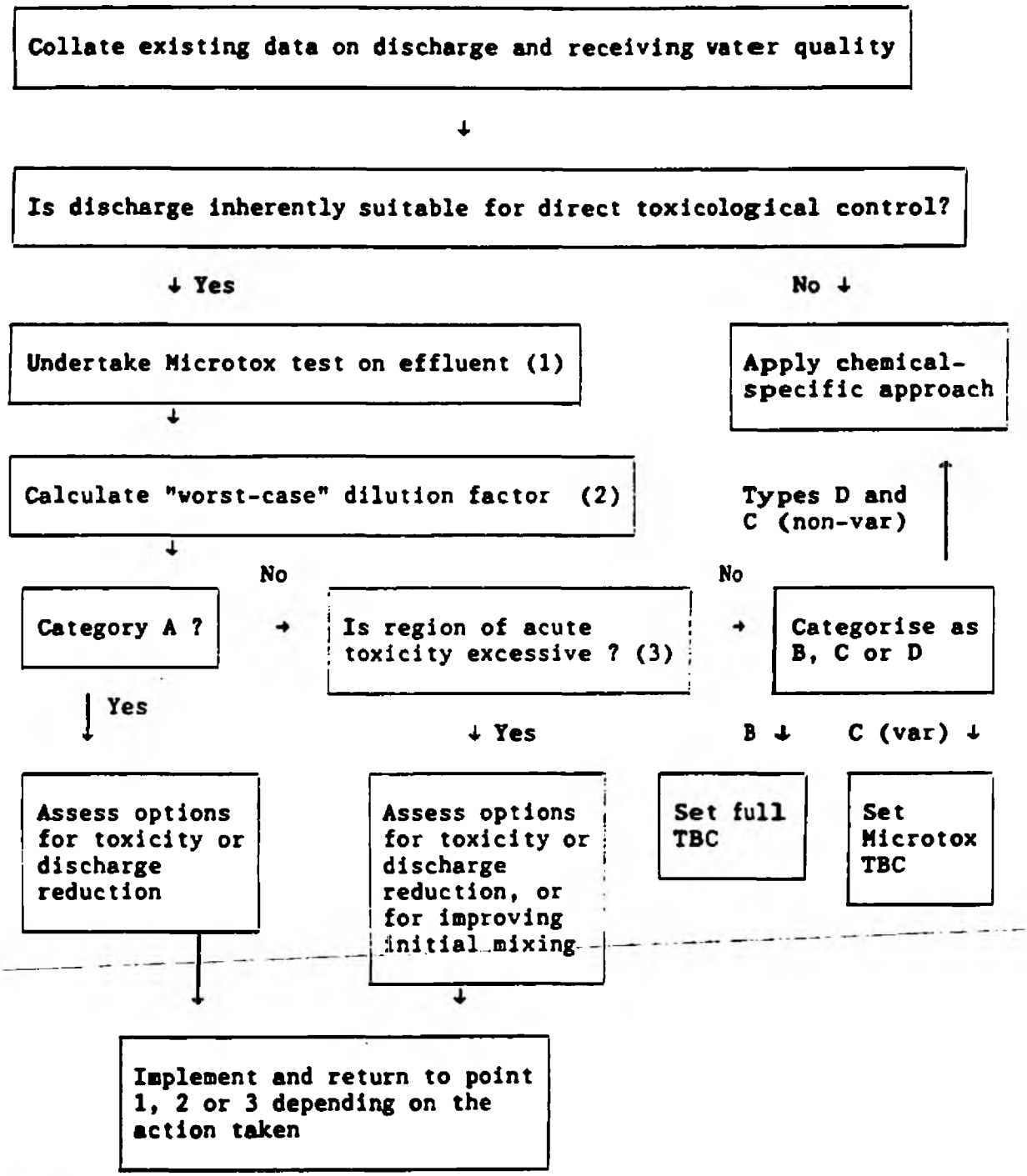
$$CIDF = 10 \times EMAT / 0.3$$

The 0.3 factor is to convert the LC50 into an LC1, to ensure that 99% - not just 50% - of the organisms are protected from lethal effects[5].

19. The size and location of the region around the discharge in which this level is exceeded (ie in which the dilution is less than that implied by the Critical Initial Dilution Factor) can then be estimated and a decision reached as to whether or not it is acceptable.

20. If it is not, steps can be taken to: (1) reduce the toxicity of the discharge, (2) reduce the discharge itself and/or (3) improve initial mixing (eg by fitting a diffuser on the outfall). Depending on the course of action taken, the toxicity of the discharge would be re-assessed using the Microtox (1), or the initial dilution recalculated (2,3), and the steps of paragraphs 18 and 19 repeated.

Figure 1. Flow chart for the application of toxicity testing to effluent control



Key to Discharge Categories:

A - Estimated Chronic In-Stream Toxicity > 1.0 TU
B - Estimated Chronic In-Stream Toxicity > 0.1 TU but <1.0 TU
C - Estimated Chronic In-Stream Toxicity >0.01 TU but <0.1 TU ("var" and "non-var" refer to variability or otherwise of effluent composition)
D - Estimated Chronic In-Stream Toxicity <0.01 TU

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